REMARKS

With this amendment, Claims 1-6 are pending. Claims 7-34 have been canceled without prejudice. Claims 1-6 have been amended.

Claim 1 has been amended to recite a method of "isolating" undifferentiated cells "that primarily differentiate into an epiblast of a blastocyst from a population of undifferentiated cells comprising: (a) sorting the population of" undifferentiated cells according to the presence or absence of "PECAM-1 on the surface of said cells; and (b) collecting the undifferentiated cells that express PECAM-1 on their cell surface, wherein the undifferentiated cells collected differentiate into an epiblast of a blastocyst at a higher rate than control undifferentiated cells." Support for this amendment can be found in the Specification in Example 5, at pages 13-14 and at page 3, line 26 - page 4, line 2.

Claim 2 has been amended to recite wherein the "population of undifferentiated cells are mouse cells and said cells further express" SSEA-1. Support for this amendment can be found throughout the Specification and, in particular, in Examples 1-5.

Claim 3 has been amended to recite wherein the "population of" undifferentiated cells are embryonic stem cells derived from mammals or embryonic germ cells derived from mammals. Support for this amendment can be found throughout the Specification and, in particular, at page 3, lines 5-14 and page 6, lines 17-21.

Claim 4 has been amended to recite wherein the "population of" undifferentiated cells are transgenic "mouse cells". Support for this amendment can be found throughout the Specification and, in particular, at page 3, lines 5-14.

Claim 5 has been amended to depend from Claim 1 and to recite wherein "the presence or absence of PECAM-1 on the surface of the undifferentiated cells is detected by the binding of an" antibody to PECAM-1. Support for this amendment can be found in the Specification at page 3, lines 13-18 and Example 1.

Claim 6 has been amended to depend from Claim 2 and to recite wherein "the expression of SSEA-1 on the surface of said mouse cells is detected by the binding of" an antibody to SSEA-1. Support for this amendment can be found in the Specification at page 3, lines 13-18 and Example 1.

No new matter has been added. Further remarks are set forth below.

Information Disclosure Statement

On the copy of the Form PTO-1449 filed April 23, 2004 that was attached to the Office Action of March 1, 2006, the Examiner crossed out reference AR (Furusawa, T. *et al.*, 2002) and wrote "Not present". However, a copy of reference AR was submitted with the Form PTO-1449 filed on April 23, 2004, and this is demonstrated by the fact that Applicants were able to download reference AR, including its concise explanation in English, from the image file wrapper of the application on public patent application information retrieval (PAIR) on the USPTO website. As a courtesy, Applicants are providing herewith a copy of reference AR downloaded from the USPTO website and a clean copy of the Form PTO-1449 submitted on April 23, 2004. Applicants request that the Examiner consider reference AR, initial the copy of the Form PTO-1449 to indicate that reference AR has been considered and return the initialed Form PTO-1449 with the next Office Action. Applicants would also like to direct the Examiner's attention to the Declaration of Dr. Tokunaga, filed under 37 CFR 1.132 with the IDS, Form PTO-1449 and reference AR on April 23, 2004, in view of reference AR.

Rejection of Claims 1-7 Under 35 U.S.C. § 112, First Paragraph - Enablement

Claims 1-7 have been rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the enablement requirement. The Examiner acknowledges that "[t]he specification has provided guidance correlating to sorting of mouse ES cells that express PECAM-1 and SSEA-1". (Office Action at page 3, paragraph 1). However, the Examiner states that "the specification has failed to provide guidance correlating to sorting of undifferentiated cells according to expression of PECAM-1, SSEA-1, SSEA-3 and SSEA-4. It is unpredictable, given variability of expression of PECAM-1, SSEA-1, SSEA-3 and SSEA-4 across species, if undifferentiated cells can be sorted based on expression patterns of PECAM-1, SSEA-1, SSEA-3 and SSEA-4." (Office Action at page 3, paragraph 1). The Examiner cites Thomson (U.S. Patent No. 5,843,780) stating the reference teaches "species-specific differences in expression of SSEA-1, SSEA-3 and SSEA-4." The Examiner also cites Cui *et al.* (*Journal of Histochemistry* 52(11): 1447-1457, 2004), stating that Cui *et al.* teaches that "within subpopulation of mouse ES cells variability exists with respect to marker expression. ...heterogeneity was evident for PECAM-1 and SSEA-1 distribution". (Office Action at page 5). Further, the Examiner states

that "it is unpredictable if expression markers such as PECAM-1, SSEA-1, SSEA-3 and SSEA-4 is limited to undifferentiated cells" (Office Action at page 5) and cites Kannagi *et al.* (*EMBO* 2(12):2355-2361, 1983), stating that the reference reports that "SSEA-3 and SSEA-4 are expressed on the surface of human teratocarcinoma cells. ... that SSEA-1 is expressed during the differentiation of teratocarcinoma cells. ... (and) that SSEA-4 is expressed on human erythrocytes, which are differentiated cells." (Office Action at page 6).

The Examiner also states that "[w]ith respect to injection of ES cells into a blastocyst for creating a chimeric embryo, it is well known that such technology is limited to ES cells obtained from mouse" (Office Action at page 3) and cites Hochepied *et al.* (*Stem Cells*, 22:441-447, 2004) to support this conclusion ("Transmission of the genotype to the offspring of chimeras has only been achieved with *M. musculus* ES cells, limiting targeted mutagenesis using ES cells to this species"). (Office Action at page 4). The Examiner further states that the germline contribution of mouse-derived ES cells is undeveloped and unpredictable even within various strains of inbred mice and cites Schoonjans *et al.* (*Stem Cells*, 21:90-97, 2003) as reporting that the "efficiency of derivation of germline-competent ES cell lines from inbred mouse strains, with specific genetic backgrounds, is greatly strain dependent." (Office Action at page 4). The Examiner goes on to state that "given the undeveloped state of the art with respect to availability of ES cells from species other than mouse, it would have required undue experimentation for one skilled in the art to make and use the invention as claimed without [sic] a reasonable expectation of success." (Office Action at page 4).

ES Cell Marker Expression

Thomson *et al.* teaches that primate (human and non-human) embryonic stem (ES) cells express the cell surface markers SSEA-3, SSEA-4, TRA-1-60, TRA-1-81 and alkaline phosphatase and that mouse ES cells express SSEA-1, but not SSEA-3, SSEA-4, TRA-1-60 or TRA-1-81 (see at col. 10, lines 13-15 and lines 29-32). Thomson *et al.* also teaches that human ES cells can be isolated by the same procedures as those described for the isolation of non-human primate ES cells (see at col. 8, lines 63-67 and col. 9, lines 23-29). Thomson *et al.* is silent regarding the expression of PECAM-1 on the surface of primate or mouse ES cells. The Specification of the instant application teaches that PECAM-1 is specifically expressed on the

surface of undifferentiated mouse ES cells that differentiate into an epiblast of a blastocyst (see Examples 4 and 5). Accordingly, Claim 1 has been amended to recite "sorting the population of undifferentiated cells according to the presence or absence of PECAM-1 on the surface of said cells". Further, Levenberg *et al.* (*Proc. Natl. Acad. Sci.* 99(7):4391-4396, 2002), reference AX on the Supplemental Information Disclosure Statement submitted herewith, teaches that PECAM-1 is also expressed on the surface of human ES cells. Thus, there are not species-specific differences in the expression of PECAM-1, the cell surface marker used to sort a population of undifferentiated ES cells in the claimed method.

Cui *et al.* teaches that there is cell-cell heterogeneity in the expression of the cell surface markers SSEA-1, PECAM-1 and ICAM-1 among undifferentiated cells in ES cell colonies (see Abstract). However, this disclosure of Cui *et al.* does not demonstrate that the practice of Applicants' claimed method is unpredictable. The teachings of Cui *et al.* indicate exactly the premise upon which the claimed invention is based (i.e., the heterogeneity of ES cell surface marker expression) and, by sorting this heterogenous population of ES cells appropriately, is precisely the problem which the claimed invention solves. Thus, it is stated in the Specification that "ES cells are not a uniform population of cells, but rather a mixture of cells with varying potential for differentiation and chimera formation. Based on this concept, the present inventors premised that fractionation of cell subpopulations would enable comparison between completely undifferentiated cells, and cells which have just begun to differentiate into specific cell lines. The inventors also concluded that fractionation would be of use in elucidating the pluripotency maintenance mechanism and differentiation induction factors in ES cells." (Specification at page 3, lines 6-12). The application Specification discloses the invention and discovery that PECAM-1 is specifically expressed on undifferentiated ES cells that primarily differentiate into an epiblast of a blastocyst (see Examples 4 and 5). Therefore, Cui *et al.* does not teach that practice of the claimed method is unpredictable; instead, Cui *et al.* demonstrates the need for the claimed invention.

Kannagi *et al.* teaches that SSEA-3 and SSEA-4 are glycolipids expressed on the surface of human teratocarcinoma cells and that, during differentiation of these cells, SSEA-3 and SSEA-4 expression decreases while SSEA-1 expression increases (see at page 2355, col. 2 paragraph 2 and Abstract). Teratocarcinoma cells (and cell lines) are derived from a type of

tumor in which germ cells have become embryonic stem cells; these cells can occur spontaneously, or can be experimentally produced. The teratocarcinoma tumor itself is comprised of an undifferentiated stem cell population that has biochemical and developmental properties similar to those of normal ES cells (Graham, 1977). Although they are not "normal" ES cells, the undifferentiated teratocarcinoma stem cells start out in the same differentiation state as normal ES cells and are used as an experimental model system for mouse and human embryo development/differentiation, as they are more readily available than ES cells (see Kannagi *et al.* at page 2355, col. 2, paragraph 1 and page 2359, col. 1, paragraph 1). The differentiation state of teratocarcinoma cells is highlighted in Cui *et al.*, in which it is stated that SSEA-1 is "expressed in preimplantation mouse embryos beginning at the 8-cell stage and also in teratocarcinoma stem cells and ES cells, but not in their differentiated derivatives. ...SSEA-1 is regarded as an excellent cell surface marker to monitor early stages of embryogenesis and ES cell differentiation." (Cui *et al.* at page 1448, col. 1, paragraph 1, citations omitted, emphasis added). Moreover, Claim 1 has been amended to recite "sorting a population of undifferentiated cells according to the presence or absence of PECAM-1 on the surface of said cells" and dependent Claim 2 has been amended to recite wherein "the population of undifferentiated cells are mouse cells and said cells further express SSEA-1." There are no teachings in Kannagi *et al.* regarding the expression of PECAM-1 on the surface of teratocarcinoma stem cells or ES cells and, further, Cui *et al.* and Thomson teach that SSEA-1 is an excellent marker for undifferentiated mouse ES cells. Therefore, the disclosure of Kannagi *et al.* does not indicate that the cell surface markers used in the claimed method (i.e., PECAM-1 and SSEA-1) are expressed on normal differentiated cells, nor that, consequently, practice of the claimed method would be unpredictable.

ES Cell Derivation and Use

Hochepied *et al.* teaches that ES cells can be derived from hybrid blastocysts of *Mus musculus* and *Mus spretus* and that the ES cells derived can be used to generate chimeric mice that transmit the *Mus spretus* genotype and phenotype to offspring. It is taught in Hochepied *et al.* that this method is a way to genetically manipulate *Mus spretus*, "as an alternative to *Mus musculus*" (see Abstract, col. 2). Hochepied *et al.* further discloses that "ES cells can be derived from many different mammalian species, including mouse, rat, rabbit and humans" (at page 44,

col. 2, paragraph 1). Thus, Hochepied *et al.* teaches the exact opposite of the statement made by the Examiner, that is, that the state of the art with respect to the availability of ES cells from species other than mouse is undeveloped. Moreover, the fact that, at the time of the invention, the technology to achieve germline transmission of ES cells from animals other than mouse was not well-established, is irrelevant to the claimed invention. Applicants' claimed method relates to isolating undifferentiated cells that primarily differentiate into an epiblast of a blastocyst from a population of undifferentiated cells by sorting the population of undifferentiated cells according to the presence or absence of PECAM-1 on the surface of the cells. Accordingly, based on the guidance in the Specification and his knowledge of the art, one of skill in the art could make and use the claimed invention without undue experimentation. The use of the undifferentiated cells after their isolation is immaterial to the practice of the claimed method and, further, there are other uses for the isolated ES cells besides the creation of a chimeric embryo/animal. For example, undifferentiated ES cells from a number of species (e.g., rat, mink rabbits, hamster, sheep, cattle, pig and human) have been used in regenerative medicine to grow tissues, skin or other cells for transplantation into a patient. (See also Specification at page 8, line 26 - page 9, line 19). Thus, undifferentiated cells isolated by the claimed method could be utilized in the same manner, in addition to their use in the creation of chimeric mouse embryos.

Schoojans *et al.* teaches a method of using of a novel cell culture medium that allows for the "efficient derivation and maintenance of ES cell lines from all 10 inbred mouse strains tested, including some that were presumed to be nonpermissive for ES cell derivation" (see Abstract col. 1, emphasis added). This disclosure by Schoojans *et al.* is also contrary to the Examiner's statement that the derivation of germline-competent ES cell lines from various inbred strains of mice is unpredictable. Instead, the work disclosed in Schoojans *et al.* demonstrates methods by which a stated problem (i.e., the difficulty of ES cell derivation in different inbred mouse strains) has been solved.

Therefore, none of the above-mentioned references cited by the Examiner teach that Applicants' claimed method is unpredictable. As amended, the instant claims are enabled by the application Specification, coupled with the existing knowledge of the art, such that one of skill in the art could practice the claimed invention without undue experimentation. As such, amended

Claim 1 and dependent Claims 2-6 meet the requirements of 35 U.S.C. § 112, first paragraph with respect to enablement. Claim 7 has been canceled, thereby making the rejection moot with respect to that claim.

Rejection of Claims 1-3 Under 35 U.S.C. § 102(b)

Claims 1-3 have been rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Thomson (US Pat. No. 5, 843,780). The Examiner states that "Thomson taught methods of isolating (sorting) primate ES cells based on the presence of cell-surface markers, SSEA-3 and SSEA-4, and the absence of cell-surface marker, SSEA-1. The presence or absence of the cell-surface markers was determined by binding of an antibody." (Office Action at page 7).

In order to make a rejection under 35 U.S.C. § 102, a prior art reference must anticipate each and every element of the claimed invention. "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir 1987).

As discussed previously, Thomson teaches that primate ES cells express the cell surface markers SSEA-3, SSEA-4, TRA-1-60, TRA-1-81 and alkaline phosphatase but do not express SSEA-1. The expression of these cell surface proteins was detected using a number of antibodies specific to each protein (see Thomson at col. 9, line 55 - col. 10, line 2). Thomson claims a method of isolating a primate embryonic stem cell line, the method comprising the steps of "(a) isolating a primate blastocyst; (b) isolating cells from the inner cell mass of the blastocyst of (a); (c) plating the inner cell mass cells on embryonic fibroblasts, wherein inner cell mass-derived cells masses are formed; (d) dissociating the mass into dissociated cells; (e) replating the dissociated cells on embryonic feeder cells; (f) selecting colonies with compact morphologies and cells with high nucleus to cytoplasm ratios and prominent nucleoli; and (g) culturing the cells of selected colonies." (Thomson at col. 22, claim 9).

The instant application teaches that PECAM-1 is specifically expressed on undifferentiated cells that differentiate into an epiblast of a blastocyst at a higher rate than undifferentiated cells that do not express PECAM-1 (see Specification Examples 4 and 5 at page

12, line 26 - page 14, line 8). Claim 1 has been amended to recite a method of "isolating undifferentiated cells that primarily differentiate into an epiblast of a blastocyst from a population of undifferentiated cells comprising: (a) sorting the population of undifferentiated cells according to the presence or absence of PECAM-1 on the surface of said cells; and (b) collecting the undifferentiated cells that express PECAM-1 on their cell surface, wherein the undifferentiated cells collected differentiate into an epiblast of a blastocyst at a higher rate than control undifferentiated cells."

Thomson does not teach, disclose or claim the isolation of undifferentiated cells that primarily differentiate into an epiblast of a blastocyst. Further, Thomson does not teach, disclose or claim the isolation of these undifferentiated cells by sorting them through the expression of PECAM-1 on their cell surface. Thus, Thomson does not anticipate any of the elements of the claimed invention. Accordingly, amended Claim 1 and dependent claims thereof (Claims 2-6) are novel over Thomson and, as such, meet the requirements of 35 U.S.C. § 102(b). Claim 7 has been canceled, thereby obviating the rejection with respect to that claim.

Information Disclosure Statement

An Supplemental Information Disclosure Statement (SIDS) is being filed concurrently herewith. Entry of the SIDS is respectfully requested.

CONCLUSION

Reconsideration and withdrawal of the rejections are requested. In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Kamilah Alexander

Kamilah Alexander
Registration No. 57,210
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133
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